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Supplemental information

Proteomic and metabolomic profiling of urine uncovers immune responses in patients with COVID-19

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Proteomic and metabolomic profiling of urine uncovers immune responses in patients with COVID-19

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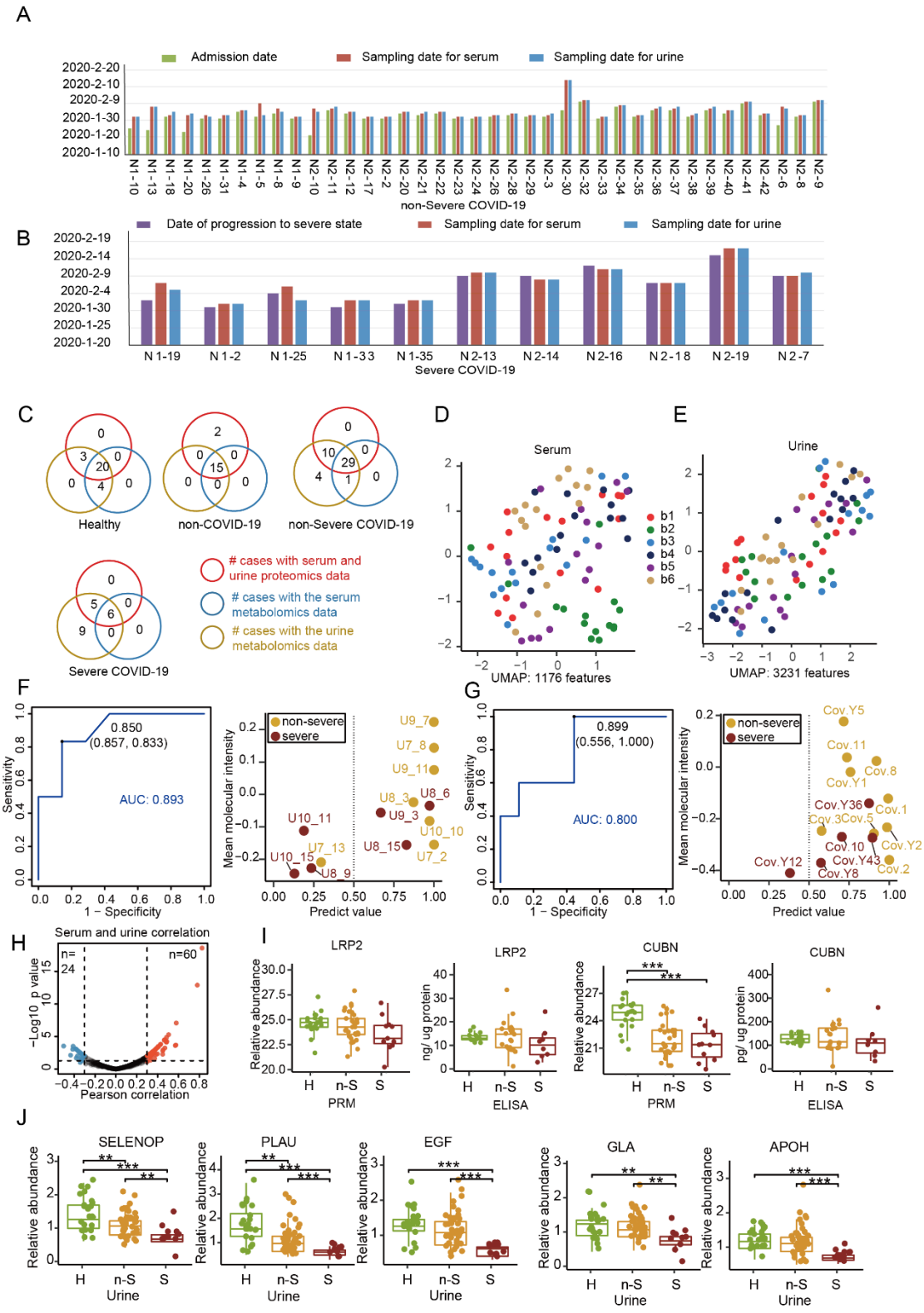


Figure S1. Sampling date information of COVID-19 cases and quality control analysis of proteomics data. Related to Figures 1, 2 and Table 1. (A, B) The admission date and sampling date for each patient in the non-severe group (A) and the severe group (B). (C) Venn diagram shows cases number of the healthy, non-COVID-19, non-severe and severe COVID-19 groups in the serum or urine proteomics and metabolomics datasets. (D, E) UMAP analysis of the (D) serum and (E) urine proteomics data of patients with COVID-19. Dots in different colors represent samples from different

batches. (F, G) Receiver operating characteristic (ROC) of the random forest model and performance of the model in the independent TMT-labelled test set of 13 patients with COVID-19 (F) and the label-free DIA data set of 14 patients with COVID-19 (G). (H) The Pearson correlation coefficient distribution of overlapped 1195 proteins in serum and urine. (I) The LRP2 and CUBN abundance in the urine quantified by ELISA and PRM method. (J)The relative abundance of SELENOP, PLAU, EGF, GLA, and AOPH in the urine.

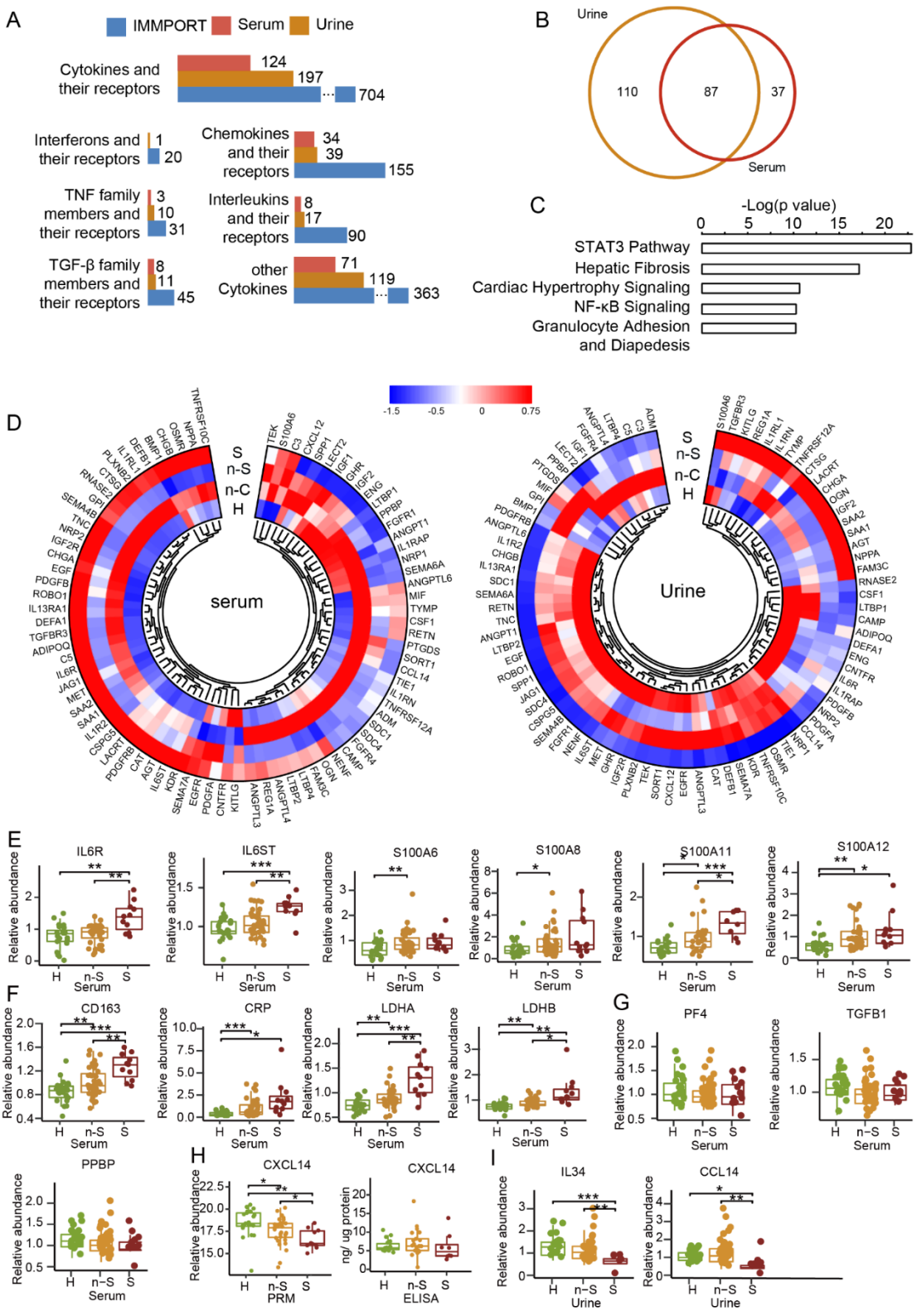


Figure S2. Cytokine analysis in the serum and urine. Related to Figure 3. (A) The number of cytokines and receptors identified in the urine, serum, and the IMMPORT database. (B) Venn diagram shows the unique and overlapped cytokines quantified in the serum and urine. (C) Regulated pathways enriched using COVID-19 associated cytokines by IPA. (D) Expression of the 87 common cytokines in the serum and urine in four groups. (E-G) The relative abundance of IL6R, IL6ST, S100A6, S100A8,

S100A11, S100A12 (E), CD163, CRP, LDHA, LDHB (F), PF4, TGFB1, and PPBP in the serum (G). (H) The CXCL14 abundance in the urine quantified by ELISA and PRM method. (I) The relative abundance of IL34 and CCL14 in the urine.

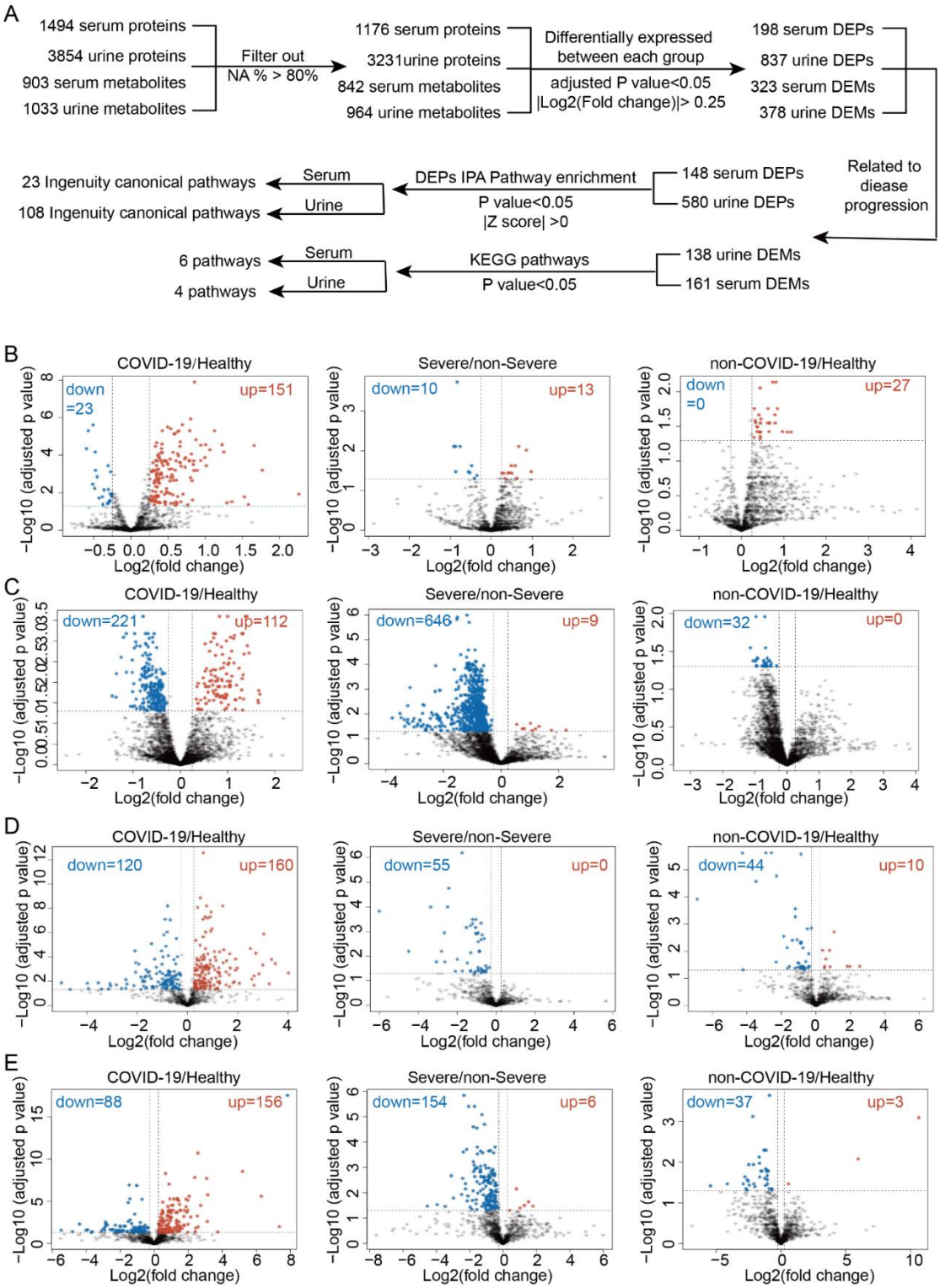


Figure S3. Differentially expressed protein analysis of proteomics and metabolomics data. Related to Figure 4. (A) The scheme for proteomics and metabolomics data analysis. Volcano plots shows DEPs in the serum (B) and urine (C), and DEMs in the serum (D) and urine (E) between COVID-19/healthy groups, severe/non-severe groups, and non-COVID -19/healthy groups. The counts (n) of significantly upregulated and downregulated molecules are shown on the top.

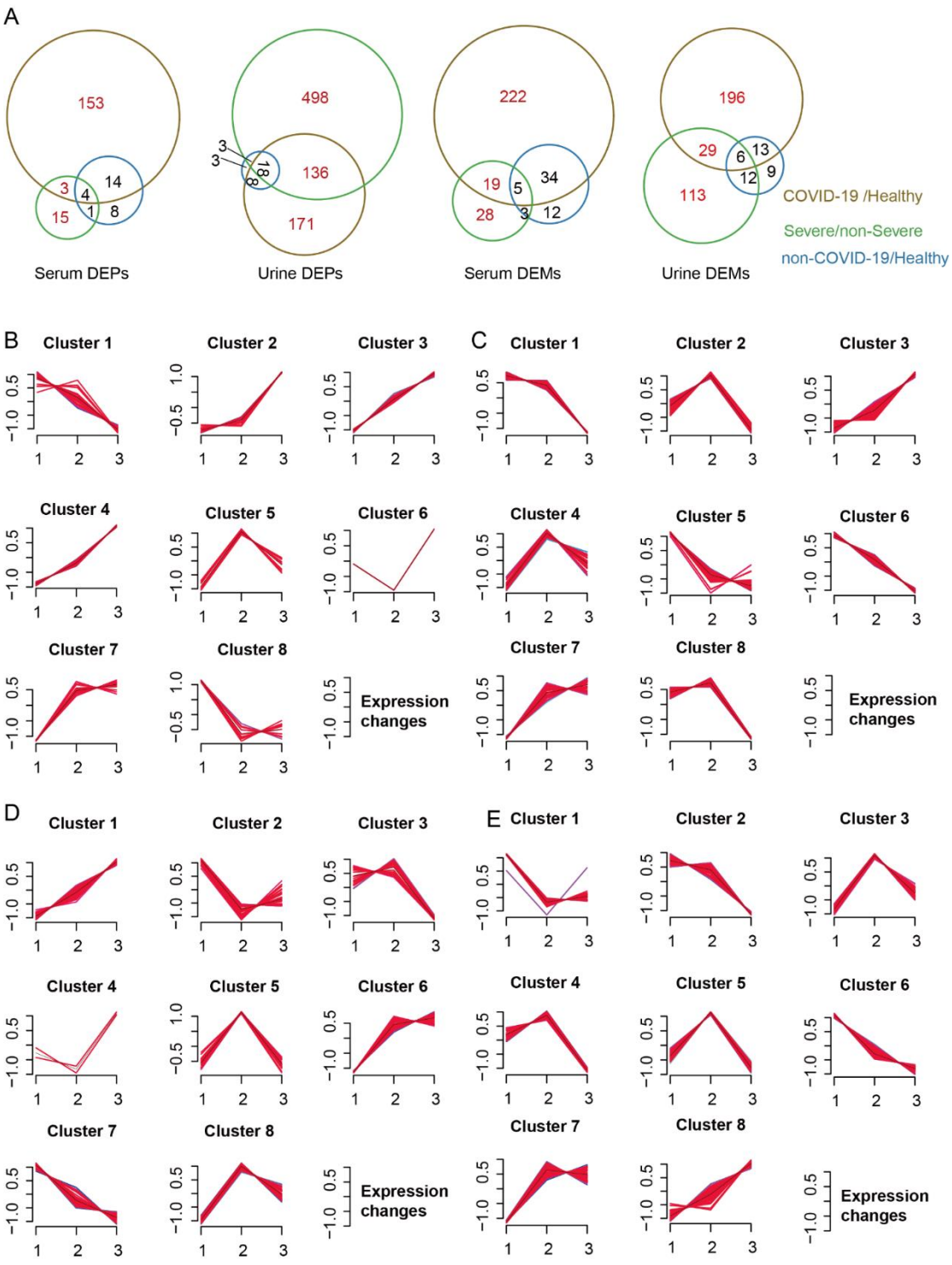
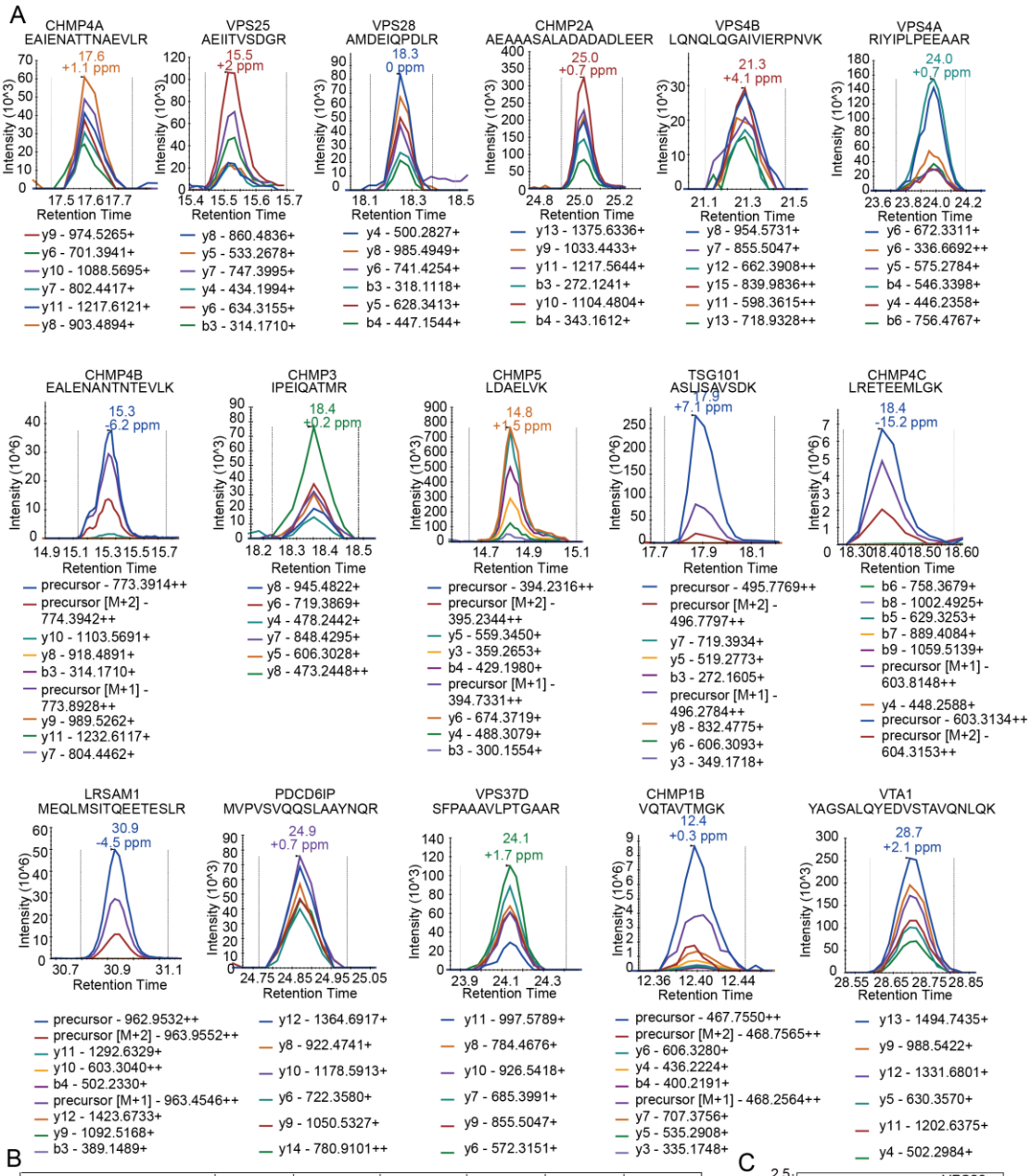


Figure S4. Expression pattern of DEPs and DEMs in the healthy, non-severe, and severe groups. Related to Figure 4. (A) Unique and overlapped DEPs and DEMs in the serum and urine. Numbers in red color are the number of DEPs or DEMs related to patients with COVID-19 after excluding the influence of the non-COVID-19 groups. Mfuzz clustering of DEPs in the serum (B) and the urine (C), and DEMs in the serum (D) and urine (E). Note: the number in x-axis: 1, healthy; 2, non-severe COVID-19; 3, severe COVID-19.



Protein	TSG101	PDCD6IP	CHMP2A	VPS4B	VPS25	CHMP5
p-value	1.4E-10	5.6E-10	6.4E-09	7.1E-09	7.4E-09	2.8E-08
Spearman correlation	0.69	0.62	0.64	0.66	0.62	0.66
Protein	CHMP4A	VPS37D	VTA1	VPS4A	CHMP3	CHMP4C
p-value	2.0E-06	2.1E-05	3.7E-05	2.3E-03	2.0E-02	4.9E-02
Spearman correlation	0.53	0.52	0.52	0.42	0.33	0.17
Protein	CHMP1B	VPS28	CHMP4B	LRSAM1		
p-value	1.5E-07	1.6E-07	8.9E-02	1.3E-01		
Spearman correlation	0.64	0.60	0.39	-0.06		

Figure S5. 16 Virus budding related proteins verified by PRM. Related to Figure 4. (A) One unique peptide was selected for each protein for PRM analysis. The selected transition groups for quantification of each peptide are shown. (B) Correlation of the quantitative results of 16 viral budding-associated proteins using both TMT and PRM methods. (C) The correlation between virus budding related proteins abundance in urine and the cycle threshold (CT) of SARS-CoV-2 reverse transcription polymerase chain reaction (RT-PCR) tests from pharyngeal swabs.

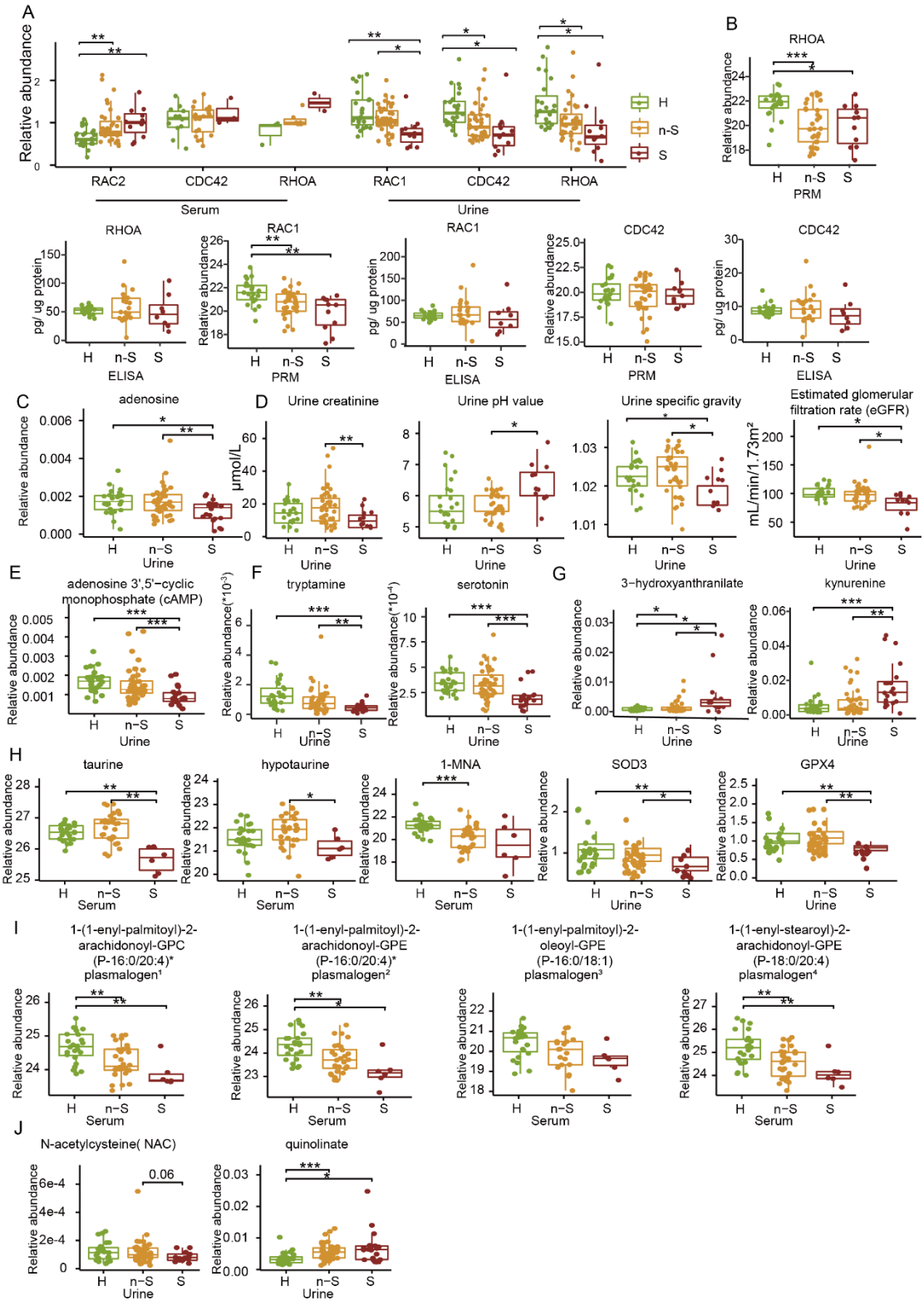


Figure S6. Proteins and metabolites involved in RhoGTPase balance and oxidative stress. Related to Figure 4. (A) The relative abundance of RHOA, RAC1, RAC2, and CDC42 in the serum and/or urine were quantified by TMT method. (B)The RHOA, RAC1, CDC42 abundance in the urine were quantified by ELISA and PRM method. (C) The relative abundance of adenosine in the urine. (D) Routine urine test of urine creatinine, pH, specific gravity, and glomerular filtration rate in healthy donors and COVID-19 cases. (E-J) The relative abundance of cAMP (E), tryptamine, serotonin (F),

3−hydroxyanthranilate, kynurenine (G), taurine, hypotaurine, 1-MNA, SOD3, and GPX4 (H), plasmalogen (I), NAC and quinolinate (J) in the serum and/or urine.

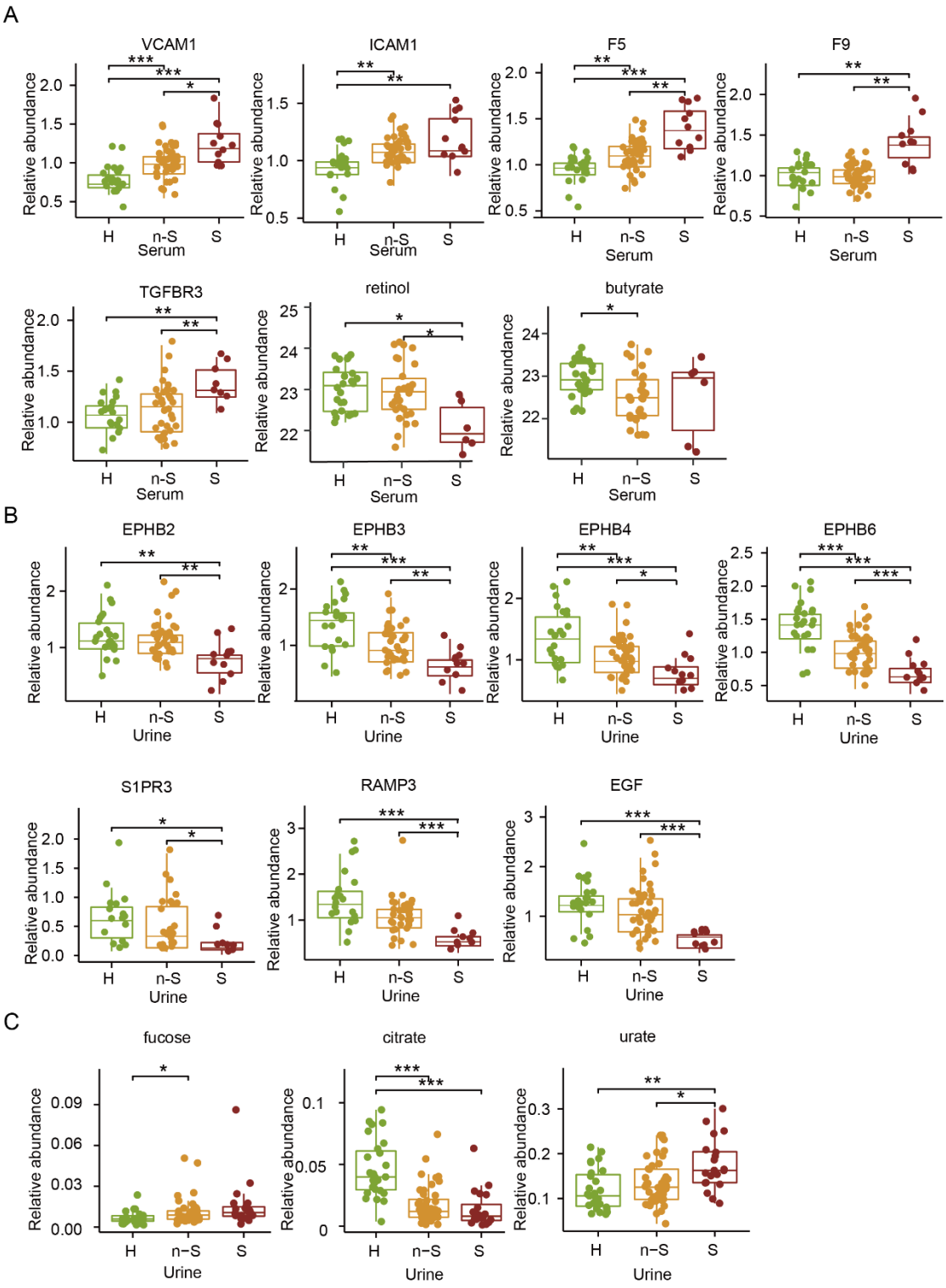


Figure S7. DEPs and DEMs related to inflammation induce renal injury. Related to Figure 5. (A) The relative abundance of potential renal injury related serum DEPs and DEMs, including VCAM1, ICAM1, F5, F9, TGFBR3, retinol and butyrate. (B, C) The relative abundance of potential renal injury related urine DEPs (B) including EPHB2, 3, 4, 6, SIPR3, RAMP3, EGF, and urine DEMs (C) including fucose, citrate and urate.